

The University of Chicago

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II. ORTHOPHENANTHROLINE AS ACCELERATOR OF BRAIN
TISSUE OXYGEN CONSUMPTION

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PHYSIOLOGY

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FRIEDA IRENE PANIMON

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IRON INDUCED OXIDATIONS IN BRAIN AND OTHER TISSUES¹

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THREE FIGURES

INTRODUCTION

Iron has long been recognized as a catalyst of oxidations by peroxide (Fenton, 1899; Stirling, '34) and by oxygen. Its role in the oxidation of lecithin and of cysteine and other sulfhydryl compounds was shown by Thunberg ('11) and Sakuma ('23) and most recently by Bernheim and Bernheim ('39). Iron aids the oxidation of fructose in the presence of phosphate (Meyerhof and Matsuoka, '24), of sulfite (Gerendas, '38), and of a number of organic molecules (Wieland and Franke, '28). Warburg ('14) found that iron, about 0.01 mg. per ml. of suspension, increased the oxygen uptake of sea urchin eggs.

The iron present in tissues has been extensively studied (McFarlane, '34), and the varying amounts in particular parts of the nervous system, 14 to 67 mgm.%, has been estimated histochemically (Spatz, '22) and by chemical methods (Wuth, '23). The reduction of ferric ion has been used as a histochemical index of neural respiration (Sugar and Gerard, '38); and an inactivation of the catalytic iron of brain tissue has been reported in association with general paresis (Hadidian and Hoagland, '39). Rosenthal and Voegtlin ('31, '33) and Voegtlin, Rosenthal, and Johnson ('31) studied the effect of iron compounds (ferric citrate and sodium tartrate, ferrous ammonium sulfate, methemoglobin, oxyhemoglobin, and the sodium salt of hematin), along with sulfhydryl and cyanide derivatives, on scissor minced rat testes, Jensen rat sarcoma, and yeast. Their early results were negative or dubious as to a catalytic action of iron on oxygen uptake, but later they found such an action, especially by ferrous salts, on the oxidation of phospholipins mainly.

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A preliminary report of this material was presented in the Am. J. Physiol., 129, P141, 1940.

We are not aware of any further study of the action of iron on tissue oxygen uptake, since Warburg's early discovery, except for the work of Bernheim and Bernheim ('39), carried on at the same time as our own studies. Related observations, on the reduction of iron by food phospholipins and phosphoproteins, have been recently reported by Tompsett ('40). Our data indicate that any action of iron on tissue oxygen uptake must be considered in terms of two separable components of the oxygen uptake, respiration and oxidation.

METHODS

In vitro determinations of oxygen consumption of brain tissue were carried out in Warburg manometers. White rats were decapitated and the cerebral hemispheres rapidly removed and stripped of their meninges and blood vessels. The brain tissue was minced with scissors until reduced to a homogeneous brei fine enough to be sucked into a pipette with an orifice less than 0.3 mm. in diameter. (Respiration of chopped cortex is approximately the same as that of slices; Holmes, '30; Stare and Elvehjem, '33). This brei was then suspended in Ringer's solution, unbuffered or buffered with acetate or phosphate (Sorensen standard); or occasionally in undiluted phosphate buffer. The Ringer's solution contained 0.1480 M NaCl, 0.0024 M KCl, 0.0018 M CaCl_2 , 0.0023 M NaHCO_3 ; or, to avoid CO_2 liberation by acid iron salts, the bicarbonate was replaced by equivalent NaCl. In more recent experiments, homogenization (Potter and Elvehjem, '36) was substituted for the scissor technique. The 2.0 ml. total fluid volume in each 18 ml. Warburg vessel was composed of 1.6 ml. brei, 0.2 ml. added solution, and 0.2 ml. N/2 NaOH (with a filter paper roll for CO_2 absorption) in the inset. Temperature was 37°C., rate of oscillation 100 per minute over a distance of 6 cm.

Ferric chloride, ferrous chloride, and ferrous ammonium sulfate were used in concentrations of 0.062 M. (3.5 mg. Fe/ml.) or some simple fraction of this. Oxidation of ferrous salts, before addition to the brei, was avoided. The high acidity of the iron solutions made pH control a major difficulty. Standard phosphate buffer, pH 7.3, was used in M/75 or M/50 or even in M/15 concentration, but complex formation with the ferric ion limits its availability. Acetate buffer, M/30 at pH 7, therefore, was also used with ferric salts. The pH of the solution in each flask at the conclusion of a run was determined with a glass electrode.

Tissues other than brain were reduced to a brei, as described above, and were suspended in Ringer or buffer in the following concentrations:

Liver, 1:20; spleen, 1:20; kidney, 1:40 or 1:60; adrenal, 1:50. Brain suspensions were generally 1:40 (sometimes 1:20) and contained 5.5 to 7 mg. of solids per ml.

RESULTS²

I. Brain

A. *Ferric ion*. 1. Unbuffered brei. Ferric ion (sublimed FeCl_3 or $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 0.004 to 0.44 mg. Fe per ml. suspension) increases the oxygen uptake of unbuffered brain brei. The Q_{O_2} curve rises rapidly for 10 or 15 minutes, falls off rather steeply during the next 15 or 20 minutes, and then declines at a gradually diminishing rate. At the end of 100 minutes oxygen usage with 0.7 mg. Fe is still two to three times greater than the control level. The extra oxygen used decreases with Fe concentration, to a negligible value with 0.004 mg. The oxygen taken up during 1.5 hours in the presence of 0.44 mg. Fe averages 4.3 times that consumed in its absence, an extra 330%³; 70 cmm. O_2 per ml. brei versus 16.

2. Phosphate-buffered brei. The bright yellow-orange FeCl_3 is promptly decolorized by phosphate addition, as a ferric complex is formed. This complex is inactive in augmenting oxygen consumption. When phosphate is added to acetate-buffered brei whose oxygen uptake is being actively catalyzed by ferric ion, it terminates the acceleration and reduces oxygen consumption to the control level, whether the pH is left at the acid value of 5 or is brought to neutrality.

3. Acetate-buffered brei. In the presence of acetate buffer, the oxygen consumption induced by ferric ion addition is more intense but less prolonged than is that in unbuffered brei (fig. 1). One hundred minutes after adding 0.44 mg. Fe per ml. brei, the oxygen consumed is approximately 125% above the control value; after 0.22 mg. Fe the excess averages 70%. With the brei homogenized, 0.22 mg. Fe is as effective as is twice that amount with the mince, producing an oxygen consumption 130% above the control figure; with 0.11 mg. Fe the excess is about 55%. The pH of unbuffered brei plus iron (2.6) is decidedly more acid than in acetate-Ringer (4.6 with 0.44, 4.9 with 0.22, and 5.4 with 0.11 mg. Fe, respectively). Since oxygen uptake induced by ferric ion is enhanced by acid, the shortened and decreased ferric action in acetate may depend upon this pH difference. There is, how-

² Results are summarized in table 4.

³ Percentage as well as absolute values are given, although the extra oxygen uptake is not directly related to the respiration, to aid comparisons between breis of different absolute content of tissue.

ever, the additional probability that a ferric-acetate complex (Wieland and Franke, '28) is less active as an accelerator than is the free ion.

4. Brei plus substrates. In phosphate buffer, M/150 glucose about doubles the brei Q_{O_2} , but ferric addition is again ineffective. In unbuffered solution, glucose steadies rather than increases brei respiration, and 0.44 mg. of Fe increases Q_{O_2} even more than in the absence of

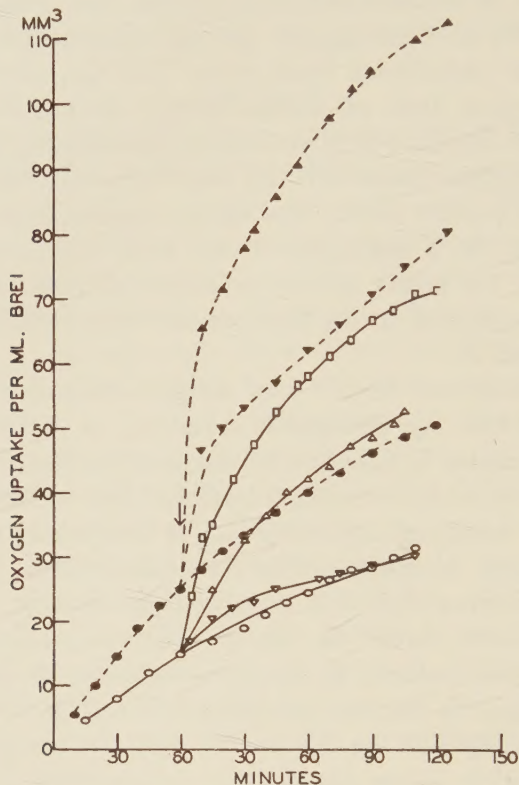


Fig. 1 Ferric effect on oxygen uptake of acetate-buffered brei. Substances were tipped at the arrow: ○, Ringer's solution tipped (control); ▽, 0.044 mg. Fe^{3+} ; △, 0.22 mg. Fe^{3+} ; □, 0.44 mg. Fe^{3+} ; ●, 0.9% NaCl (control); ▼, 0.11 mg. Fe^{3+} ; ▲, 0.22 mg. Fe^{3+} . Open symbols and solid lines represent scissor-minced brei; filled symbols and dashed lines represent homogenized brei. Curves are averages of four to twenty-one experiments each.

substrate. The Q_{O_2} is maintained at a level five times that of the control for approximately an hour. The total oxygen consumed in 100 minutes is 2.3 times the control amount.

M/150 sodium succinate triples the initial Q_{O_2} of brei in unbuffered Ringer's. Iron addition (0.44 mg. per ml., but not 0.044) leads to an oxygen consumption similar to that without specific substrate. An hour after adding iron to succinate brei, the Q_{O_2} value remains 100% above

the control; and the total oxygen consumed after 100 minutes is 45% higher. It is not improbable that ferric ion forms a complex with succinate as well as with acetate (Treadwell and Hall, '35). Malonate inhibits the utilization of succinate but, when tipped with FeCl_3 , does not interfere with the iron acceleration. One experiment in the presence of M/150 sodium lactate showed a very sharp rise and decline in Q_{O_2} after tipping FeCl_3 (0.44 mg. Fe), with oxygen usage still double the control 90 minutes later. The iron action in M/150 Na pyruvate is not significantly different from that without addition.

5. Heated, centrifuged, or extracted brei. After its residual oxygen consumption has been abolished or reduced to less than one-tenth by heat, centrifuging at 2500 R.P.M. for 10 minutes, or extraction with trichloroacetic acid or acetone, brain brei suspension still takes up oxygen under the influence of added iron. The oxygen utilized is approximately proportional to the quantity of solids present in the trichloroacetic precipitate or in the residue left by acetone extraction. More precisely, the residue after repeated acetone extraction retains 70% of the oxidizable material of the original brei, the rest being lost in processing. This residue yields two-thirds of its activity to petroleum ether and retains one-third, again with loss of about a quarter of the oxidizable material. Further fractionation of the petroleum ether extract divides activity about equally between lecithin and cephalin fractions.

6. Brei plus inhibitors. Urethane, 2.5% final concentration, inhibits the respiration of brain brei markedly but has virtually no effect on the ferric acceleration. Warburg ('14) found that the acceleration of sea urchin egg respiration by small amounts of ferrous ion is inhibited by this narcotic.

NaCN , 10^{-2} to 10^{-3} N, inhibits brain brei respiration by 80% and completely blocks the acceleration of oxygen uptake by ferric iron; 10^{-4} N cyanide inhibits neither.

Sodium pyrophosphate ($\text{Na}_4\text{P}_2\text{O}_7 \cdot 10\text{H}_2\text{O}$) is strongly alkaline and, on this basis, inhibits the respiration of brei; when neutralized to pH 7.2 its inhibitory effect is negligible. But, neutralized or alkaline, M/135 pyrophosphate does inhibit the ferric acceleration (fig. 2; compare Kharasch et al., '36).

7. Secondary additions. After the acceleratory effect of one ferric ion addition (minced brei) has begun to wear off, a second addition is relatively ineffective. This is true even when the initial iron addition was small and the acceleration far from maximal. A second addition

of brei, heated or unheated and after varying times of autoxidation, results in an oxygen uptake that includes a new iron acceleration. When only a small amount of iron (0.11 mg. per ml.) is present, however, this renewed catalysis is feeble or absent, indicating that the iron has been bound by the first brei. With homogenized brei, secondary iron additions are effective unless the first iron portion has already induced maximal oxidation. Clearly, accessibility of the oxidizable systems, in

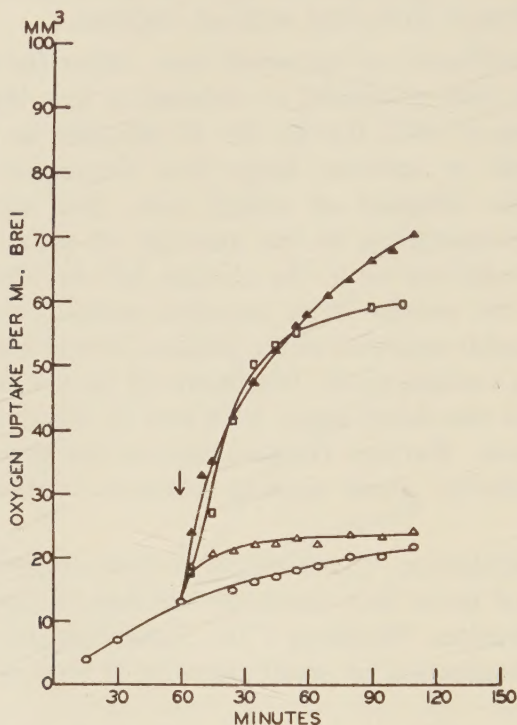


Fig. 2 Effect of sodium pyrophosphate on ferrous and ferric catalyses. ○, Ringer's solution tipped (control); △, 0.44 mg. Fe^2 ; □, 0.44 mg. Fe^2 ; ▲, 0.44 mg. Fe^3 . Open symbols represent additions in the presence of M/135 pyrophosphate; the filled symbol represents Fe^3 addition in the absence of pyrophosphate. For Fe^2 the curves in the presence and absence of pyrophosphate coincide.

terms of particle size or dispersion, as well as of iron, is important in these oxidations.

8. Controls. Control experiments included the tipping of H_3PO_4 and HCl to check acidity effects, and of NaCl to check osmotic effects; and the addition of the ferric salt to the medium with or without substrate but without brei. The extra oxygen consumption did not appear in any case. Acidification of homogenized brain brei to pH 4.9 or 5.3,

with HCl rather than FeCl_3 , inhibits respiration 40 to 50% within 2 to 3 hours. The respiration of spleen and of kidney brei is even more acid sensitive.

B. Ferrous ion. 1. Unbuffered brei. Ferrous ion, in the same molar concentrations as ferric ion, was added as ferrous ammonium sulfate ($\text{FeSO}_4(\text{NH}_4)_2\text{SO}_4 \cdot 6\text{H}_2\text{O}$) or ferrous chloride ($\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$). 0.44, 0.044, or 0.004 mg. Fe per ml. increases the oxygen consumed by brei in Ringer during 100 minutes by 110%, 33%, and 30% respectively.

2. Acetate-buffered brei. Ferrous ion oxidizes to ferric slowly in acid solution, more rapidly as neutrality is approached; so that in acetate buffer (M/30, pH 4.7) oxidation of 0.7 mg. Fe (1.6 ml. containing 0.44 mg. per ml.) is not complete even after 2 hours, in phosphate (pH 7.0) it is finished within an hour. (In 3% sodium acetate, pH 5.9, oxidation is ended in 45 minutes.) With 0.35 mg. Fe in acetate buffer (pH 5.0), oxidation is virtually complete at the end of the second hour. In only one experiment of twelve with 0.44 mg. per ml. was any oxygen used beyond that needed for ferrous oxidation; but half this amount of iron did cause an extra oxygen uptake, average 16 cmm. per ml., in eight out of thirteen runs. One positive result was obtained with brei heated for 45 minutes at 70°C . to kill respiration and one with minced brei; the others were with homogenized, respiring brei. There is some evidence that, although variable iron action is encountered between pH 5 and 6, acceleration is consistent and large above or below this range—e.g., at pH 4 or 7. No extra CO_2 production accompanies the extra oxidations induced by iron.

3. Phosphate-buffered brei. In M/15 phosphate buffer or M/60 phosphate-buffered Ringer, ferrous chloride is oxidized and the calculated amount of oxygen, 100 cmm. per mg. Fe^{2+} , is taken up within an hour, 90% within the first $\frac{1}{2}$ hour. Readings after addition of iron to brei were appropriately corrected. The corrected total oxygen consumption in 2 hours, of minced tissue in M/15 phosphate, is increased to 150%, 135%, 95%, 50%, and 17% above the control by the addition, respectively, of 0.44, 0.22, 0.11, 0.044, and 0.004 mg. of iron per ml. (fig. 3). 0.22 mg. Fe^{2+} exerts an almost maximal effect, double this concentration does not significantly increase the rate or amount of oxygen utilization (table 1). With homogenized brei, 0.11 mg. iron leads to the same increased oxygen usage as with minced tissue, but 0.22 mg. leads to a greater one—180% instead of 135%.

The concentrations of brei and of phosphate have been varied independently; since, on the one hand, the rate of multimolecular reactions falls more than proportionally with dilution (Krebs, '35; Potter and

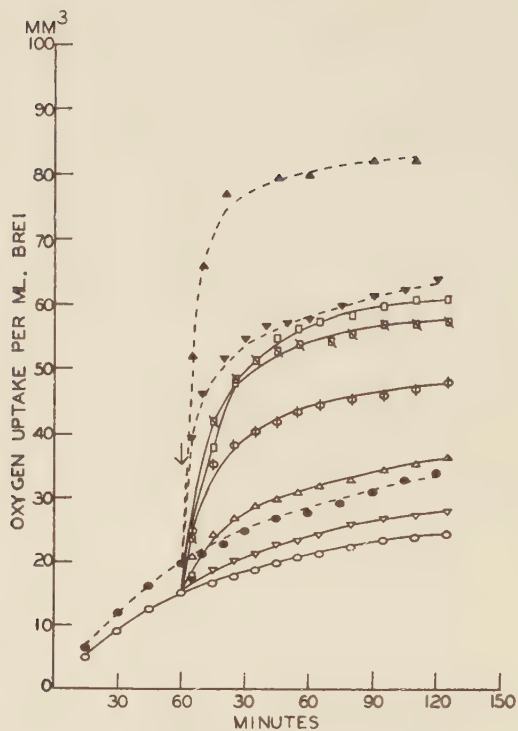


Fig. 3 Ferrous effect on brain brei in M/15 phosphate buffer. $\circ$, phosphate or 0.9% NaCl tipped (control); ∇ , 0.004 mg. Fe^{2+} ; $\triangle$, 0.044 mg. Fe^{2+} ; $\diamond$, 0.11 mg. Fe^{2+} ; $\square$, 0.22 mg. Fe^{2+} ; $\square$, 0.44 mg. Fe^{2+} ; $\bullet$, 0.9% NaCl (control); $\blacktriangle$, 0.11 mg. Fe^{2+} ; $\blacktriangle$, 0.22 mg. Fe^{2+} . Open symbols and solid lines represent scissor-minced brei; filled symbols and dashed lines represent homogenized brei. Curves are averages of two to fourteen experiments each.

TABLE 1

Brain (1:40 brei). Action of FeCl_2 in PO_4 (M 1/15). Influence of Fe concentration

ADDITION MG. Fe^{2+} /ML. BREI	CMM. O_2 /ML. BREI 2 HOURS AFTER TIPPING	CMM. O_2 /ML. FeCl_2 (NO BREI) Observed	Theoretical	O_2 UPTAKE CORRECTED FOR Fe^{2+} OXID.	CORRECTED O_2 UPTAKE AS PER- CENT OF CONTROL
....	24			24	100
0.44	105	45	44	61	255
0.22	79	20	22	57	240
0.11	58	10	11	47	195
0.04	40	4	4	36	150
0.004	28.5	0	0.4	28	117

Elvehjem, '36) and, on the other hand, M/15 phosphate interferes with some oxidations. Actually, a 1:20 suspension of brei in M/15 phosphate consumes twice as much oxygen as does a 1:40 suspension, and the iron effect per ml. of suspension is also double (table 2). Also a 1:40 brei behaves in M/15 phosphate as it does in M/60 phosphate, although the latter is more acid—pH 6.6 with 0.22 mg. Fe added.

Since, in a phosphate medium, Fe^{2+} ion is soon completely oxidized and added Fe^{3+} ion is inactive, the question arises: How can Fe^{2+} be effective? Two solutions of FeCl_2 in M/60 phosphate-saline were allowed to autoxidize at 37°C . until 85% and 30%, respectively, of the iron was oxidized. The first was added to brei to give 0.044 mg. of iron per ml. (0.006 mg. Fe^{2+}); the second to give 0.22 mg. of iron per ml.

TABLE 2

Brain. Action of FeCl_2 in PO_4 . Influence of concentration of brei and PO_4 .

ADDITION MG. Fe^{2+} /ML. BREI	BREI CONC.	PO_4 CONC.	CMM. O_2 /ML. (CORRECTED) IN 2 HOURS AFTER TIP.	O_2 UPTAKE WITH Fe^{2+} IN PER CENT OF CONTROL
Ringer	1:40	M/60	23	100
Ringer	1:40	M/15	20	100
Ringer	1:20	M/15	40	100
0.004	1:40	M/60	27	115
0.004	1:40	M/15	24	120
0.004	1:20	M/15	48	120
0.22	1:40	M/60	65	280
0.44	1:40	M/15	60	300
0.44	1:20	M/15	110	275

(0.156 mg. Fe^{2+}). As controls, 0.044 and 0.22 mg. of unoxidized ferrous ion were used. The oxygen consumed was proportional to the ferrous ion present and independent of the total amount of iron (table 3). Titration of Warburg vessel contents with ceric sulfate indicated that the ferrous catalysis, in a phosphate medium, ceases when oxidation of the added iron is completed.

4. Brei plus inhibitors and accelerators. NaCN , 10^{-3} N, does not affect ferrous catalysis appreciably, ten times this concentration depresses it by about 40%.

Pyrophosphate forms a very stable ferrous complex (Michaelis and Friedheim, '31); yet, in the presence of M/135 pyrophosphate, ferrous addition exerts its usual influence on brei oxygen usage (fig. 2). Thus, in 100 minutes, brei with pyrophosphate used 21 cmm. of O_2 per ml., and the same plus 0.44 mg. of iron used 60 cmm. It follows that the complex

ion has as great an activity as the ferrous ion alone. In fact, Warburg ('24) points out that free Fe^{2+} does not react with molecular oxygen.

Orthophenanthroline and ferrous iron form an extremely stable complex. Yet this dye not only fails to inhibit the ferrous acceleration, but the ferrous-phenanthroline formed is far more effective than is the ferrous ion alone or in other complexes. Actually, the dye alone in concentrations as low as 10^{-5} M. final concentration, markedly increases brei oxygen consumption. Its action is dealt with more fully in the following paper.

5. Secondary additions. After a small amount of ferrous ion (0.11 mg. per ml.) has acted upon a portion of brei, further iron addition leads to a new increase in oxygen usage (about 15% as much as would occur if the added iron were present at the start) but addition of fresh brei is without effect. A single iron addition produces the same oxygen

TABLE 3

Brain (1:40 brei). Action of FeCl_2 in $\text{PO}_4 \left(\frac{M}{60} \right)$. Influence of state of oxidation of Fe

MG. Fe ADDED	pH	CMM O_2 /1.6 ML. BREI	
		Calculated	Observed
0	7.15		13
0.35 Fe^{2+}	6.62		121
0.35 { 0.25 mg. Fe^{2+} 0.10 mg. Fe^{3+}	6.71	90	91
0.07 Fe^{2+}	7.04		46.5
0.07 { 0.01 mg. Fe^{2+} 0.06 mg. Fe^{3+}	7.10	17.5	21.5

uptake whether added to fresh brei or to brei which has autoxidized for 2 hours or more. It follows that some fixed amount of material in the brei, not one of the regular substrates, is oxidized by the iron; but that a given portion of iron can act only once. Warburg ('14) similarly found that a second ferrous addition increased the direct oxidation of tartaric acid. With a granular suspension from sea urchin eggs, however, the iron effect fell off as did the autoxidation.

If the secondarily added iron is in the ferric form (brei in acetate buffer), the ferric-ferrous combination seems to act somewhat more rapidly, and its acceleration falls off sooner, than does the ferric ion alone.

C. *Other metals.* Copper, manganese, and zinc are without effect on the oxygen uptake of the brain preparation used; nickel and cobalt may inhibit slightly.

II. Other tissues

A. *Ferric and ferrous ions.* Liver, kidney, and spleen were minced and suspended in Ringer to form breis of 1:20, 1:60, and 1:20 concentrations, respectively; or a weighed amount of tissue was homogenized in the appropriate medium. FeCl_3 was tested in acetate buffer, FeCl_2 , in phosphate. The total oxygen uptake of liver, as of brain, is markedly increased by both ferric and ferrous ions. Fractionation of liver brei indicated that 30% of the oxidizable material is protein.

TABLE 4
Brain, 1:40 brei. Summarized results

BUFFER	SUBSTRATE	SPECIAL TREATMENT	IRON ADDED	NUMBER EXPERIMENTS AVERAGED	QO_2				$\text{MM}^3 \text{O}_2$ USED	
					1 hour average	At tip	Maximum after tip	Time of maximum in minutes	In 90 minutes after tip	In excess of control total
....				28	12	8.5			6	
			Fe^3	40			100	20	65	70
			Fe^2	18			15	35	20	20
		Heated	Fe^3	3	0	0	70	25	70	70
Acetate				30	14.5	10.5			14	
			Fe^3	29			115	At once	52	44
			Fe^2	15			75	At once to 30 min.	40	35
Phosphate				17	14.5	9.5			8.5	
			Fe^2	33			250	At once to 10 min.	45	37
....	Glucose			1	18.5	15			19	
			Fe^3	4			110	15	70	60
		Heated	Fe^3	2	0	0	50	40	50	50
....	Succinate			17	32	22.5			23	
			Fe^3	25			65	At once	48	29
			Fe^2	13			27	At once	21	0
		Heated	Fe^2	1	0	0	50	At once	45	45

The oxygen usage of spleen, on the contrary, is inhibited by ferric ion (equivalent acidification with HCl causes a similar depression), but is somewhat accelerated by the ferrous salt. Both minced and homogenized preparations of kidney respond to the ferrous ion with an increased oxygen consumption (possibly due to decrease of a phosphate inhibition); while ferric ion inhibits the minced but accelerates the homogenized tissues. The oxygen uptake of kidney mince narcotized with urethane is, however, increased by Fe^3 . One experiment with adrenal tissue indicated an acceleration by ferric ion.

B. Buffers. The various buffers strongly influence tissue brei respiration, aside from any action on added iron. Brain and spleen mince respire alike in acetate or phosphate, but liver respiration is greater in the latter medium and that of kidney about 40% lower. Respiration of homogenized brain (as that of homogenized kidney) is maintained better in acetate. Phosphate has been shown to increase fumarate oxidation by brain (Quastel, '31) and the respiration of nerve (Shaffer, Chang and Gerard, '35), but to depress the respiration of paramecium (Gerard and Hyman, '31), of rat liver and kidney (Van Heyningen, '35; see also Warburg, '31), and of muscle and heart (Stare and Baumann, '36).

C. Injury. The additional mechanical manipulation attendant upon homogenizing the tissue depresses the respiration of kidney markedly, but not that of spleen or of brain. The respiration of scissor-minced kidney is three to four times greater than that of the homogenized tissue and does not fall off as rapidly. Homogenized spleen and brain respire somewhat better than the minced preparations and, at least with brain, maintain respiration much better.

D. Effect of spleen on brain response. Spleen brei oxidation is not accelerated by ferric ion or by orthophenanthroline or its ferrous complex. When added to brain brei, spleen produces, roughly, a 50% inhibition of the oxidation increase achieved by dye addition in the absence of spleen. A suspension of erythrocytes inhibits similarly; plasma has no effect.

DISCUSSION

Under appropriate conditions, addition of iron salts to minced or homogenized tissue preparations leads to a greater uptake of oxygen, as much as four times greater, than would occur without iron addition. In the case of ferrous ion, which is itself oxidized at rates depending on pH, anions, and tissue, a correction must be made for oxygen so used; but an additional oxygen consumption still remains. The iron salts used, especially ferric chloride, are strongly acid and control of pH by reasonable amounts of buffer has been only partly successful; and specific chemical action of buffer anions is a further complication. Despite these variables, certain facts and conclusions seem clear.

The material in brei which is oxidized only after iron addition is not part of the substrate of normal respiration. It may, however, be concerned in the respiration mechanism since iron reduction by brain slices is quantitatively related to their rate of oxygen consumption (Sugar and Gerard, '38). The oxygen usage induced by iron is alike

in fresh brei with or without substrates added and in brei which has autoxidized for hours. It is not eliminated by heat, narcosis, precipitation, or extraction which abolishes residual respiration; and it is accompanied by no CO_2 liberation. At an acidity which decreases or stops respiration, the ferric action is present and rapid. The respiration or the iron action can be differentially blocked by such inhibitors as cyanide or pyrophosphate. The oxidizable material is precipitated by trichloroacetic acid and is not extracted by acetone, two-thirds of it is subsequently extracted by petroleum ether and appears to be a mixture of lecithin and cephalin; the remainder is left behind, indicating its protein nature. Bernheim and Bernheim ('39) have precipitated such oxidizable proteins from rat liver and trout eggs and have related their behavior in part to free SH groups. Since their material was inactive after acid treatment or acetone extraction, and responded poorly to iron alone; and because of our negative results on the catalytic oxidation of cysteine by orthophenanthroline, it is not certain that we are dealing with the same systems. The nitroprusside test was consistently negative at the end of a run on our controls as well as on the iron treated samples—indicating that oxidation of free SH groups cannot alone account for the excess oxygen used.

The maximum extra oxygen usage induced by iron in brain brei was about 10 cmm. per mg. dry weight. Since this value was attained under varying conditions, it probably measures the total oxidizable material. As further evidence for this is the inability of a second iron addition to increase oxygen consumption, after a maximal action by the first iron added. Under many experimental conditions, however, oxygen use ends long before the oxidizable material has been exhausted. This is due to a progressive unavailability of reactants. A special case is the change of ferrous iron to an inactive ferric state. More generally, binding of iron as a complex with phosphate, protein, etc., or its actual removal in a coagulum; or the similar loss of substrate from suspension or solution as a result of coagulation by acid or iron, serves to end the oxidation. Related to the latter, is the degree of destruction of cells and entry into intact ones of the added agents. The facts, that a second addition of iron to homogenized but not to minced tissue renews oxygen uptake following incomplete oxidation, and that a second addition of brei is not acted upon by iron previously present in limited amounts, support these conclusions.

A further consideration is the discrimination between the normal metabolic oxidation of substrates, for which we reserve the term "respiration," and a specially catalyzed oxidation which is different

from the usual respiration. The respiration of controls is subtracted from the oxygen consumption of the iron treated samples to obtain the catalyzed oxidation; but when this respiration (residual or with substrates) is considerable and is itself partly destroyed by the added acid iron salts, a serious error is possible. Thus, in the case of kidney mince, ferric addition markedly decreases oxygen usage. But the same addition to mince with its respiration decreased by urethane, or to homogenized brei with a respiration reduced by the greater breakdown to one-fourth that of the mince, leads to an increased uptake of oxygen.

The ferric inhibition of spleen respiration is a similar case, but with the important difference that this ion (and ferrous orthophenanthroline as well) fails to catalyse oxidations in this tissue. Further, the presence of spleen—or of erythrocytes or hemoglobin, but not of blood plasma—inhibits the ferric (and dye, but not ferrous) action on brain brei.

The buffer anions have specific actions, aside from the use of phosphate mainly at pH 7.0 and of acetate at pH 5.0 (after iron addition). The respiration of brain brei, as of spleen, is alike in both buffers; that of liver is considerably greater in phosphate, while the reverse is true for kidney. The action of ferric ion, added as such or formed during a run by oxidation of ferrous iron, is blocked by phosphate, perhaps enhanced by acetate. Ferrous ion acts well in phosphate but is partly suppressed by acetate. Pyrophosphate and cyanide, rather like orthophosphate buffer, block ferric ion but not ferrous. A discussion of the mechanism of iron catalysis, to which these data are relevant, is included in the following paper.

SUMMARY

The oxygen consumption of brain and other tissue breis, with or without added substrate (succinate, pyruvate, glucose, lactate), is increased by addition of ferric or ferrous ion. The reaction is much influenced by pH and buffer anions.

The extra oxygen used serves to oxidize substances, probably protein (precipitated by trichloroacetic acid, thermostable, etc.) and phospholipins, not serving as substrates in normal respiration; but perhaps concerned in the respiratory mechanism. The catalyzed oxidations are not restricted to $-SH$ groups.

Ferric action is inhibited by ortho- and pyrophosphate and by cyanide, not by urethane. Catalysis is best in acetate buffer.

Ferrous ion, although itself oxidized, catalyzes most effectively in phosphate buffer. Its action is not inhibited by the above agents or by others which form stable complexes with it.

Both iron ions catalyze oxidations in liver and kidney breis as in brain. Ferric ion is inactive on spleen, and spleen (or hemoglobin) inhibits the ferric induced oxidations of brain.

Other metals (Cu, Mn, Zn, Ni, Co) fail to increase oxygen usage.

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ORTHOPHENANTHROLINE AS ACCELERATOR OF BRAIN TISSUE OXYGEN CONSUMPTION¹

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FOUR FIGURES

I. INTRODUCTION

The catalytic action on cell respiration of a number of dyes has been discovered and studied (e.g., methylene blue, Harrop and Barron, '28; pyocyanin, Friedheim, '34; Young, '37; thionine and brilliant cresyl blue, Dickens, '36; see also Barron, '39); and their effect on brain respiration in schizophrenics is being investigated (Gerard, '38, and unpublished). We here report still greater accelerations of oxygen uptake obtained with orthophenanthroline and its ferrous complex.

Ferrous orthophenanthroline, first described by Blau (1898), has been used as an oxidation-reduction indicator since the work of Walden, Hammett and Chapman ('31). Over the pH range in which it has been employed (pH 0 to ca. 3) its E_o' value has been established at +1.14 (or +1.06, Shaffer, '39). The ferrous form, a vivid red in color, is resistant to oxidizing agents (Cambi and Cagnasso, '34; Shaffer, '39) and, at room temperature, is only very slowly decomposed by strong acids or substituted by other metals that form stable complexes with the base (Walden, Hammett and Chapman, '33).

Phenanthroline and α, α' dipyridyl, which are similar in ferrous complex formation, have both been utilized to remove free iron (Ustvedt, '33; Zuckermandl and Messiner-Klebermass, '33; Hill, '30). Ferrous dipyridyl, as also the free dye, catalyzes the autoxidation of linoleic acid (Franke, '32), and activates cathepsin (Michaelis and Stern, '31).

The present study of phenanthroline grew out of the use of this compound in an attempt to inhibit, by complex formation, the ferrous ion acceleration of brain tissue oxygen consumption (Panimon, Horwitt and Gerard, '41). Not only did this dye fail to inhibit the ferrous effect,

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but, alone or as the iron complex, it proved to be a potent (oxidation) accelerant.²

II. METHODS

The oxygen uptake of homogenized (Potter and Elvehjem, '36) suspensions of cerebral brei, before and after the addition of orthophenanthroline or the ferrous orthophenanthroline complex, was measured in Warburg manometers. Details of technique were as described (Panimon, Horwitt and Gerard, '41).

Orthophenanthroline monohydrate ($C_{12}H_8N_2 \cdot H_2O$) was dissolved in water, and 0.2 ml. added to 1.6 ml. of brei suspension. The final concentration of the dye ranged from 10 mM/liter to 0.002 mM/liter. To effect solution of the highest concentration used, 0.5 ml. of N/2 CH_3COOH was added per 10 ml. water.³

The ferrous-phenanthroline complex was prepared either by dissolving the appropriate amount of crystalline monohydrate in a ferrous chloride solution, or by mixing measured quantities of metal salt and dye already in separate solution. The complex is very soluble. It was used in final concentrations of one-third the molarity of the free dye, since three moles of phenanthroline combine with one of iron. Other metal complexes of orthophenanthroline were prepared similarly, with proper allowance for the number of dye molecules bound to each metal ion.

With the buffer concentration used (Sorensen phosphate, $\frac{M}{15}$ or $\frac{M}{60}$) pH, determined with a glass electrode system, remained fairly uniform at 7.3 in all experiments.

III. RESULTS

A. Orthophenanthroline

The optimal concentration of the free dye, 0.2 mM/liter, increases by six times the rate of oxygen usage by brain brei during the first hour after the addition (Q_{O_2} 42 versus 7 per cc. brei; about 0.17 times these figures per mgm. dry weight). The rate falls to, and remains at, that of the control after an extra 45 cmm. of oxygen has been taken up, in 2 hours. During this period, the dye treated brain consumes four

² Both the brown and the blue ferric complexes are unstable and were not used. The former, resulting from the direct combination of phenanthroline and $FeCl_3$, adds additional phenanthroline molecules in solution and forms less stable complexes (Gaines, Hammett and Walden, '36). The blue salt, formed by oxidation of the ferrous compound, is light sensitive, being quickly reduced in sunlight.

³ Blau states that the solubility of α, α' dipyridyl in water is 1 part in 200, while for its ferrous complex the figures are 1 part in 4. For orthophenanthroline the same relationships hold.

times as much oxygen as does the untreated, 59 versus 14 cmm. Lower dye concentrations led to lesser increases in rate, but these were longer maintained, so that the total extra oxygen used did not fall off as rapidly as did the rate of consumption. Even 10^{-5} M dye concentration doubled the oxygen used during 2 hours. When the optimal dye concentration was exceeded, the acceleration was progressively less with increasing dye addition until, at about 10 mM/liter, augmentation of



Fig. 1 Orthophenanthroline effect on oxygen uptake of brain brei. Substances were tipped at the arrow: ○, 0.9% NaCl tipped (control); △, 0.02 mM/L orthophenanthroline; □, 0.2 mM. orthophenanthroline; ▽, 2.0 mM. orthophenanthroline.

oxygen consumption passed over to mild depression. The curves in figure 1, based on six to twenty-eight experiments each, show the course of oxygen uptake with no dye present, with the optimal concentration added, and with one-tenth and with ten times the optimum amount.

B. Ferrous orthophenanthroline

At equivalent molarity, 0.07 mM/liter optimal, the ferrous complex induces an extra oxygen consumption by brain brei comparable to that induced by the free dye. The initial maximal rate increase, eleven

times the control (Q_{O_2} 77 versus 7), does not hold up so long; after an hour the rate is three times the untreated brei and after 100 minutes the rates become and remain equal. Concentrations below optimal give feeble but more prolonged accelerations, as in the case of the free dye; but those above optimal behave somewhat differently in the two cases. The iron complex in ten times optimal concentration gives a somewhat greater initial acceleration, Q_{O_2} about 100, but oxygen usage ceases in about 90 minutes, when an excess of 38 cmm. above the control

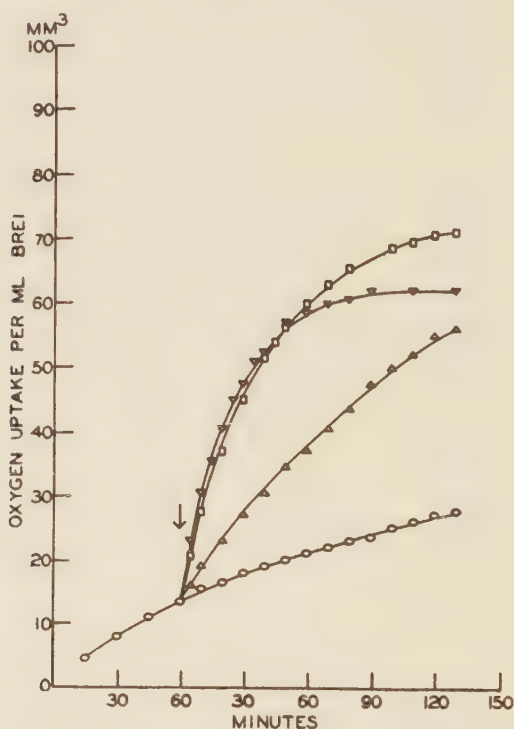


Fig. 2 Ferrous orthophenanthroline effect on oxygen uptake of brain brei. $\circ$, 0.9% NaCl (control); $\triangle$, 0.007 mM/L Fe^{2+} -orthophenanthroline; $\square$, 0.07 mM Fe^{2+} -orthophenanthroline; ∇ , 0.7 mM Fe^{2+} -orthophenanthroline.

has been consumed. At no concentration tested (3.4 mM/liter maximum) did ferrous orthophenanthroline fail to accelerate oxygen uptake. The curves in figure 2, based on seven to twenty-eight experiments each, show the action of the ferrous complex and are comparable to those in the first figure.

The actions on brain brei of optimal amounts of dye, of the iron-dye complex, and of a quantity of ferrous ion ten times that in the complex, are compared in figure 3. It is clear that the ferrous complex is a much

more powerful accelerant than is the free ferrous ion; also that the dye action initially lags behind that of the complex while the ferrous ion action is most prompt. Q_{O_2} values are summarized in table 1.

The more detailed study of these oxidation accelerants is in progress; but it can now be stated that addition of a substrate (0.2% glucose) does not alter the action of the dye or of its complex, nor does the presence of urethane or previous heating of the brei. Further, although the R.Q.

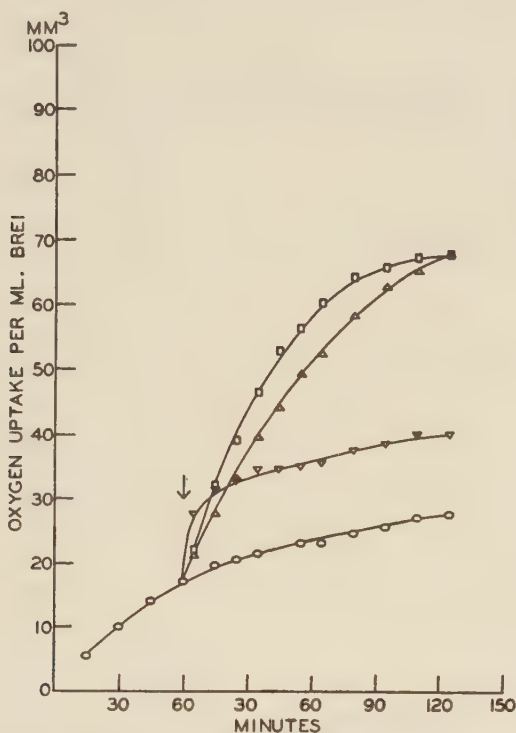


Fig. 3 Comparison of effects of ferrous iron, orthophenanthroline, and ferrous orthophenanthroline on oxygen uptake of brain brei. ○, 0.9% NaCl (control); ∇, 0.044 mg. Fe^{2+} ; △, 0.2 mM/L orthophenanthroline; □, 0.07 mM. Fe^{2+} -orthophenanthroline (= 0.004 mg. Fe^{2+} added).

of the residual respiration is 1.0, as is also that of the increase induced by thionine (confirming Dickens, '36), no extra CO_2 is formed under the action of the phenanthroline molecules. Oxygen consumption of brei from liver, kidney, or diaphragm is increased by these additions much as that of brain brei, while a spleen preparation is not only not accelerated itself but depresses the acceleration with other tissues. Hemoglobin, as well as a suspension of erythrocytes, similarly inhibits this oxidation catalysis.

TABLE 1
Brain brei in *M*/15 phosphate buffer. Summarized results

BEEI CONCEN- TRATION	SUBSTRATE	ADDITION IN MM./LITER	NUMBER EXPERI- MENTS AVERAGED	Q _{O₂}			MM. ³ O ₂ USED		
				1 hour average	At tip	Maximum after tip	Time of maximum in minutes	In 90 minutes after tip	In excess of control- total
1:40	—	NaCl, 17 FeCl ₃ , 0.07 o-phen, 10 5 2 0.2 0.02 0.01 Fe-o-phen, 1.7 0.7 0.07 0.035 0.007 0.0007	16	13.5	10	—	10	—	
			7			16	At once	13	4
			5			12	At once	16	10
			5			20	At once	22	18
			9			24	At once	27	24
			10			50	30	51	45
			6			27	40	39	34
			1			27	30	20	25
			4			84	At once	42	33
			7			115	At once	46	38
			12			77	At once	53	44
			1			47	At once	46	39
			7			32	At once	33	29
			2			20	At once	13	6
1:20	Glucose	PO ₄ , 7 o phen, 2 0.2 0.02 Fe-o-phen, 0.7 0.07 0.007	1	22	18	—	14	—	
			1			90	At once	42	38
			1			80	At once	63	49
			1			72	10	46	40
			1			180	At once	59	44
			1			90	10	64	50
			1			42	At once	36	31
			6	27	18	—	—	18	—
			2			36	At once	25	7
			7			96	At once	89	75
			6			105	At once	92	81
			6			—	—	—	—
			6			—	—	—	—

C. Role of sulfhydryl groups

Since no CO_2 is liberated by phenanthroline, the dye might act to catalyze SH group oxidation. This was studied with a pure cysteine solution (cysteine hydrochloride, purified by recrystallization from HCl), to which was added ferrous ion, ferric ion, phenanthroline, or the iron-dye complex. Purified cysteine autoxidizes very slowly, only one-fourth being oxidized at the end of 5 hours (fig. 4). Iron added as

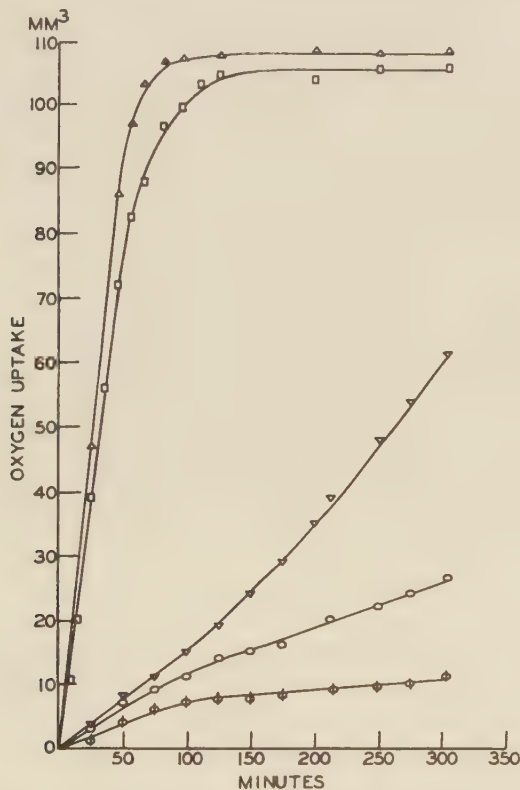


Fig. 4 Oxidation of cysteine hydrochloride (3 mg.) in the presence of ferrous iron, ferric iron, orthophenanthroline, and ferrous orthophenanthroline. ○, H_2O (control); ◊, 0.2 mM/L orthophenanthroline; ▽, 0.07 mM/L Fe^{2+} -orthophenanthroline (= 0.007 mg. Fe^{2+}); □, 0.007 mg. Fe^{3+} ; △, 0.007 mg. Fe^{2+} . 106 $\text{mm}^3 \text{O}_2$ are required for complete oxidation to cystine.

FeCl_3 or FeCl_2 (0.07 or 0.007 mg. $\text{Fe}/3$ mg. cysteine· HCl) leads to complete oxidation in 100 minutes. Orthophenanthroline alone slows oxidation, perhaps by tying up traces of iron contaminating the cysteine. The iron-dye complex is far less active than is free iron, and its slight acceleration might be due to ferrous ions released by a minimal dissociation.

D. Complexes of other metals with orthophenanthroline

The specificity of action of the ferrous complex is further indicated by comparison with the action of other metal-orthophenanthroline combinations. The colorless zinc and the colored copper, nickel, and cobalt complexes, blue, red, and yellow, respectively (Blau, 1898), as well as the free metal ions, were added to brei suspensions. None of the metal ions accelerated oxygen usage, even when added in amounts equal to the most effective iron concentrations; and, of the metal complexes, only the zinc produced an increased oxygen uptake. At the two concentrations of zinc complex tested, however (final value, 2×10^{-3} and 2×10^{-4} molar; three dye to one metal in the complex) equivalent amounts of free phenanthroline were as, or more, effective. It is interesting that the zinc tri-dipyridyl, like the iron compound, is an activator of proteolysis by cathepsin (Michaelis and Stern, '31).

E. Dipyridyl and ferrous-dipyridyl

Preliminary tests with dipyridyl and its ferrous complex indicate actions on brain brei similar to those of the equivalent phenanthrolines. The greatest acceleration obtained was with the highest concentration used, ten times the molarity of orthophenanthroline for optimal effect. The oxygen uptake during 90 minutes in the presence of dipyridyl was approximately 2.5 times the control value; with the metal complex it was almost three times the control.

IV. DISCUSSION

An increased oxygen consumption of brain, and other tissue, slices or brei, on addition of dyes in concentrations of the order of 10^{-5} molar, has been reported by several investigators (Elliott and Baker, '35; Young, '37; Dickens, '36; Dodds and Greville, '33). In most cases, the acceleration at this dye concentration was to less than double the control value, and it was considerably less or absent when no substrate was added, or when some buffer or pH other than the optimal one was chosen. With the dye systems here studied, 10^{-5} molar addition more than doubled the oxygen used by brain during an hour and greater amounts tripled it. The initial acceleration was more than ten times the control rate with the iron complex. Orthophenanthroline, however, seems to catalyze oxidations which are not a part of normal respiration.

The dye or its ferrous complex, as also ferrous or ferric ion, induces the oxidation of a substrate, largely or entirely phospholipin and protein, present in tissue brei but not entering into normal respiration.

No CO_2 results from this oxidation and the R.Q. is zero, although that of the residual respiration and of the extra oxygen consumption induced by thionine under like conditions (carbonate buffer) is 1.0. Yet the finding that orthophenanthroline actually inhibits the oxidation of cysteine, makes it less likely that SH groups are being oxidized by it in the brei. The same conclusion may apply to the experiments with unbound iron. We shall assume, for the following discussion of iron catalysis, that all extra oxygen consumption results in our experiments from oxidation of the same substances and by the same route.

Many workers have considered the form of iron which is catalytically active. An oxidation-reduction cycle between Fe^{2+} and Fe^{3+} , in free or combined form, has been demonstrated for the cytochrome system Keilin, '29) and urged as the usual mechanism (Warburg, '14; '24); but other investigations favor the view that only Fe^{2+} (Wieland and Franke, '28; Zuckerkandl and Messiner-Klebermass, '33) or only Fe^{3+} (Stirling, '34) is active in particular systems. If a cycle is involved in these oxidations, then ferrous ion must be oxidized by oxygen to ferric and this in turn reduced by substrate. Since, in phosphate, Fe^{3+} is inactive and catalysis by a ferrous-ferric mixture is proportional to the ferrous concentration only, it would be necessary to assume further that the ferric ion, newly formed from ferrous, acts immediately as oxidizer before it is effectively removed as a phosphate complex. That this is possible in a simple system is shown by adding ferric ion to orthophenanthroline in phosphate buffer, with or without ascorbic acid present as a reducing substrate. With substrate, red ferrous orthophenanthroline is formed; without it, no color appears.

On the other hand, Fe^{2+} does gradually change to and accumulate as Fe^{3+} . In phosphate, therefore, some of the newly formed Fe^{3+} must be bound as such and the amount available for oxidizing substrate must be lessened. Yet the rate of catalyzed oxidations with Fe^{2+} is greater in phosphate than in acetate or in the the absence of buffer. Nor can it be argued that in phosphate the rate of formation of ferric ion is sufficiently increased so that, even with some bound, the effective concentration of "nascent" Fe^{3+} is greater than without phosphate. The overall shift of Fe^{2+} to Fe^{3+} , in the absence of brei, is slower in phosphate at pH 5.9 than in acetate at pH 5.6; in the presence of pyrophosphate, the rates are alike in phosphate, pH 6.5, and acetate, pH 5.6. Further, oxidations produced by addition of Fe^{2+} are complete within an hour, those due to direct addition of Fe^{3+} continue for over two. This last point must be discounted, however, because of a probable influence of the different acidities in the two sets of experiments.

If the behavior in phosphate is difficult to account for in terms of an iron cycle, that in acetate is even harder to interpret. Ferric ion is active in acetate, ferrous ion changes to ferric in acetate, yet the catalytic action of ferrous ion in acetate is feeble or absent, especially at pH 5 to 6. Since ferrous acceleration is greater and more constant in stronger, as well as weaker, acid, this feeble action cannot be attributed to a destruction of tissue respiration sufficient to mask the newly induced oxygen uptake. Indeed, after the residual respiration has been destroyed by heat or other means Fe^3 is still far more effective in acetate than is Fe^2 .

Wieland and Franke ('28) found that acetate accelerated the oxidation of several pure substances by ferrous ion and suggested the formation of an active ferrous acetate complex. Although the acetate action is different with brei, such a complex may form and it is conceivable that iron oxidized in such combination forms an inactive ferric compound. Certainly addition of fresh ferric salt leads to a renewed oxygen usage by acetate brei which has completed the oxidation of an original supply of ferrous ion and of the oxidations induced by it.

This introduces the problem of iron complexes. Under our conditions, pyrophosphate, as orthophosphate, inhibits ferric but not ferrous or ferrous-orthophenanthroline catalysis. Grieg and Munro ('39) attributed the failure of pyrophosphate to inhibit the respiration of brain slices to failure of penetration into the cells; but our similar finding with homogenized brain brei opposes this interpretation. Since pyrophosphate, like malonate, inhibits the succinic but not the cytochrome systems (Leloir and Dixon, '37; Keilin, '29), it has been suggested that both act by virtue of the double acid groups and that no iron complex is involved. Yet we find pyrophosphate blocks ferric catalysis without affecting brei respiration while malonate blocks respiration with succinate without affecting iron action. Further, ferrous-pyrophosphate has been shown to be active in the formation of formic acid (Spoehr and Smith, '26). Cyanide also inhibits ferric oxidations, and respiration as well, but does not significantly disturb ferrous catalysis. Ferrous orthophenanthroline, however, is blocked by this inhibitor.

Clearly iron forms specific complexes in each oxidized state, and some of these are catalytic, others not. Ferric ion is more easily reduced to ferrous when in combination as a complex than when in ionic form (Baudisch and Welo, '24); yet there is no basis for assuming that any of these complexes depend on a valence change of the iron atom for their activity. This is perhaps most clear for the ferrous orthophenanthroline complex, which has no stable ferric form and which acts

without dissociating. Thus, ferrous ion in the complex is over 100 times as effective as in the absence of the dye, on the one hand; and on the other, orthophenanthroline complexes with cobalt, nickel, and copper are no more stable than is that with iron, yet they do not dissociate enough free dye to give any acceleration at all.

Since orthophenanthroline does form a stable complex with ferrous iron, the question arises whether the free dye acts as such or after combination with iron present in the tissue. Were the latter the case, it might help account for the more rapid onset of acceleration with the iron complex than with the free dye. It could also easily account for the fact that large concentrations of free dye, but not of its iron complex, inhibit tissue oxygen uptake; for the dye could remove iron from other essential roles. As some support for this interpretation, are the facts that dye and iron complex both produce the same maximal oxygen consumption and do so at the same optimal dye concentration. The red color of ferrous orthophenanthroline does not appear when the dye is added to brei but it would not be expected at such dilutions. If all the iron in brain were available for conversion to the complex, it would make less than half the optimal concentration of this.

Especially interesting is the anomalous behavior of spleen, erythrocytes, or hemoglobin. Neither ferric ion nor orthophenanthroline induces spleen oxidations; and hemoglobin—free, in blood cells, or in the spleen—diminishes or blocks the catalytic action on brain brei of the dye, of the iron-dye complex, or of the free ferric (but not ferrous) ion. This is reminiscent of the ability of muscle extracts specifically to inhibit the iron catalyzed oxidation of thioglycollic acid (Kharasch, etc., '36). However, this suggested interaction between iron porphyrin and iron phenanthroline complexes demands experimental rather than theoretical analysis. Preliminary spectroscopic observations did not indicate any action of the phenanthroline on oxyhemoglobin.

V. SUMMARY

1. Orthophenanthroline and ferrous orthophenanthroline accelerate the oxygen consumption of brain brei in final dye concentrations as low as 2×10^{-6} M. The optimal concentration, 2×10^{-4} M, triples oxygen uptake over a 2-hour period and initially accelerates the rate six to ten times. Added substrate (glucose) does not enhance the acceleration.

2. Cobalt, nickel, and copper complexes of orthophenanthroline produce no acceleration; the zinc complex, a small one.

3. Kidney, liver, and muscle brei oxygen consumption is accelerated much as is brain brei; that of spleen brei is not increased. Spleen, ery-

throcytes, or hemoglobin depress the positive dye action on other tissues.

4. Increased oxygen uptake is also effected in the presence of the related compound, α , α' dipyridyl, as well as its ferrous salt.

5. Ferrous orthophenanthroline acts as such, the free dye possibly after combining with tissue iron.

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